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Chemical Diversity of Scarab Beetle Pheromones and its Implication in Chemical Evolution

Jason Lorenzo Lumabas[1], John Carlos Sioson[1], Jose Isagani B. Janairo[1,2*]
[1]Biology Department, De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines;
[2]Biological Control Research Unit, Center for Environmental and Natural Sciences Research (CENSER), De La Salle University, 2401 Taft Avenue, Manila 0922, Philippines
*E-mail: jose.isagani.janairo@dlsu.edu.ph

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Abstract

Pheromones are species-specific chemical signals used by insects to communicate, to find a mate, and to identify their territory. In this paper, we analyzed the structural similarity of scarab beetle pheromones using the Tanimoto coefficient in an attempt to draw insights regarding their ecology, evolution and chemotaxonomy. The results showed a very diverse scarab beetle pheromone structure which provides further support to an earlier hypothesis regarding beetle pheromone evolution. In addition, it was found that the scarab beetle pheromone structure cannot be used as a species marker in chemotaxonomy owing to the observed high structural diversity

Keywords: Chemical Ecology, Molecular Similarity, Chemotaxonomy, Pheromones, Scarabaeida.

Introduction

The family Scarabaeidae is composed of 20 subfamilies where four of which are considered as agricultural pests (Leal 1971, 1998). The stems, leaves, nectar or pollen of flowers, and the living roots of crops are the usual targets of these beetles (Leal 1998). More so, they are known as social creatures that communicate through the use of chemical signals called pheromones. These plant-feeding beetles use such signals to respond to a species of the same kind. Functions of it include alerting other organisms of possible danger, attracting or repelling mates, and marking its territory (Harrari and Steinitz 2013). Pheromones basically serve the purpose of communicating and conveying information to similar species that is used by majority of insects (Wyatt 2003). Unfortunately, there is very limited information about these chemical signals. Only the subfamilies Melolonthinae and Rutelinae have recorded structures of sex pheromones in scarab beetles while aggregation pheromones of only two species from the subfamily Dynastinae are identified up to date (Leal 1971, 1998). Analyzing the structural diversity of the pheromones of some identified species from the three subfamilies will be critical in studying these chemical signals. Furthermore, studying the relatedness of these signal chemicals from a structural perspective may provide clues regarding how the behavior of these beetles toward semiochemicals evolved. In this paper, we present the cluster analysis based on pheromone structural similarity in an attempt to explore possible scenarios relating to the chemical evolution of the pheromones for these beetle families. Moreover, we also probe the prospect of using pheromones for the chemotaxonomic classification of these beetles. The classification of the compounds produced by these beetles would entail the creation of methods to control the agricultural damage they may bring about.

Materials and Methods

Specimen

The list of pheromones and their corresponding species was taken from Leal (1998). There are 10 beetle species namely Costelytra zealandica, Heptophylla picea, Holotrichia parallela, Holotrichia consanguinea, Anomala rufocuprea, Anomala cuprea, Anomala schonfeldti, Blitopertha orientalis, Phyllopertha diversa, and Oryctes monoceros (Table 1). Four of these species belong to the subfamily Melolonthinae, five species are under the subfamily Rutelinae, and Orcytes monoceros being the only species under the subfamily Dynastinae.

Table 1: Chemical compounds of various beetle species (Entry numbering corresponds to Table 2 and Fig.1).

Entry	Chemical Name	Species	Subfamily
1	(5S)-5-[(1E)-1-Decen-1-yl]dihydro-2(3H)-furanone	Costelytra zealandica	Melolonthinae
2	methyl 5-(Z)-tetradecenoate	Anomala rufocuprea	Rutelinae
3	(R,Z)-5-(—)-(1- octenyl)oxacyclopentan-2-one	Anomala cuprea	Rutelinae
4	2-(E)-nonenol	Anomala schonfeldti	Rutelinae
5	7-(Z)-tetradecen-2-one	Blitopertha orientalis	Rutelinae
6	1,3-dimethyl-2,4-(1H,3H)-quinazolinedione	Phyllopertha diversa	Rutelinae
7	(R,Z)-7,15-hexadecadien-4-olide	Heptophylla picea	Melolonthinae
8	pheno	Costelytra zealandica	Melolonthinae
9	(R)-(—)-linalool	H. parallela	Melolonthinae
10	anisole	Holotrichia consanguinea	Melolonthinae
11	ethyl 4-methyloctanoate	Oryctes monoceros	Dynastinae

Pheromone structure analysis

The structural similarity of the pheromones was pairwise compared using the publicly available software Small Molecule Subgraph Detector (Rashman 2009). A requirement prior to the use of the software is the translation of the chemical structures into SMILES format which was carried out using ChemSpider. The software provides the Tanimoto coefficient between the two molecules that are being compared. A score that is closest to one indicates a high degree of structural similarity. From the calculated coefficients, a similarity table (Table 2) was constructed which served as the basis for the hierarchical cluster analysis.

Table 2: Similarity table of the different chemical compounds

	1	2	3	4	5	6	7	8	9	10	11
1	1	0.68	0.88	0.62	0.76	0.11	0.62	0.10	0.35	0.14	0.38
2		1	0.58	0.53	0.58	0.11	0.70	0.10	0.35	0.14	0.38
3			1	0.67	0.87	0.12	0.60	0.11	0.39	0.16	0.35
4				1	0.67	0.14	0.53	0.0	0.47	0.0	0.41
5					1	0.12	0.68	0.0	0.39	0.0	0.35
6						1	0.10	0.40	0.09	0.38	0.12
7							1	0.09	0.38	0.13	0.41
8								1	0.12	0.88	0.11
9									1	0.12	0.33
10										1	0.17
11											1

The dendrogram was constructed using DendroUPGMA, which follows an unweighted pair group method with arithmetic mean algorithm as the amalgamation method.

Results

The calculated Tanimoto coefficients were utilized for the pair-wise comparison for the similarity of eleven identified pheromone structures produced by beetles under the Scarabaeoidea family. The results of the hierarchical cluster analysis based on structural similarity were then analyzed within the context of chemotaxonomy, ecology and evolution. The dendrogram (Figure 1) produced from the hierarchical cluster analysis exhibited high chemical structural diversity of the pheromones analyzed.

This is seen from the fact that the subfamilies under Scarabaeoidea cannot be classified according to their pheromone. This indicates that the pheromones released by the beetle species under each subfamily are structurally very different. There are only two beetles from the same subfamily, Costelytra zealandica (8) and Holotrichia consanguinea (10), have related pheromone structures that can be seen at the bottom of the dendogram. Beside the similarity of two Melolonthinae beetles, the sex pheromones from the subfamilies Melolonthinae and Rutelinae and aggregation pheromone from the subfamily Dynastinae have no distinct similarities in structure.

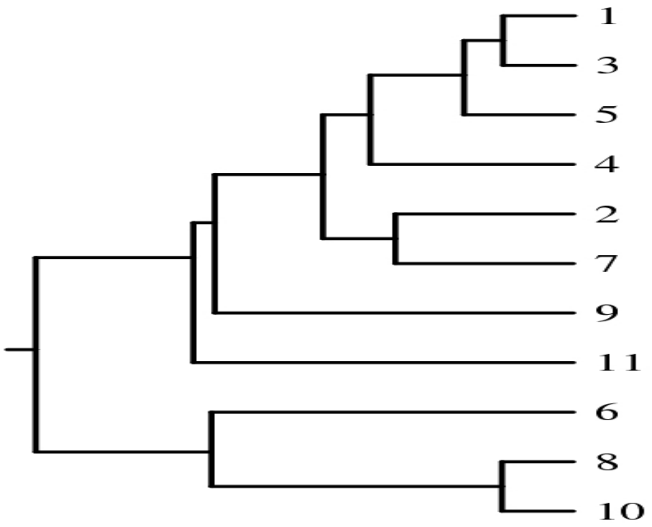

Figure 1: Dendogram of the pheromone compounds found in the beetles

Discussion

One of the most commonly used criteria in measuring molecular similarity is the Tanimoto coefficient. It identifies how similar the molecules are, depending on bit-string representations (Gupta and Kumar 2014). Decomposition into fragments is applied to the structures being studied; “1” is given to the fragments or features existent in a certain molecule, while “0” for the ones that are absent (Flower 1998). Each molecule would then have a string that contains “1”s and “0”s or bit strings. The quantification of the similarity between molecules is done through the computation of chemical coefficients as that of the Tanimoto coefficient (Flower 1998; Park et al. 2010). This metrics of similarity is usually applied in medicinal chemistry in order to look for promising compounds based on the structural similarity with drug leads.

The species Costelytra zealandica (1), Heptophylla picea (7), Costelytra zealandica (8), Holotrichia parallela (9), and Holotrichia consanguinea (10) are beetle species that belongs to the subfamily Melolonthinae. The Melolonthinae beetles Heptophylla picea and Holotrichia parallela are both distributed throughout Japan and China with the former being an endemic species of Japan.

The species Costelytra zealandica or commonly known as the grass grub is a widespread pest that is endemic to New Zealand (Unelius et al. 2008). The white grub, Holotrichia consanguinea, on the other hand, is distributed along North India and is localized in states of Rajasthan, Bihar, and Uttar Pradesh (Srivastava 2009). On the other hand, Anomala rufocuprea (2), Anomala cuprea (3), Anomala schonfeldti (4), Blitopertha orientalis (5), and Phyllopertha diversa (6) all corresponds to beetles under the subfamily Rutelinae. The three species from the genus Anomala used in the study, A. rofocuprea, A. cuprea, and A. schonfeldti are all distributed in the country of Japan with A. rofocuprea localizing in Sapporo (Matsuki et al. 1997). The other two Rutelinae beetles in the study, Blitopertha orientalis and Phyllopertha diversa are geographically distributed in Southeast Asian countries like Japan, China, and Korea. The aggregation pheromone of the sole Dynastinae beetle Oryctes monoceros (11) does not show similarity in pheromone structure with the other two subfamilies. This beetle is geographically widespread in African countries like Sierra Leone and Nigeria and causes major damage to coconut palms (Bedford 1979; Hoyt 1963).

The geographic localization of these beetle species in addition to the results of our cluster analysis support the hypothesis raised by Leal (1997) regarding the evolution pheromone communication. Leal (1997) proposed that pheromone communication evolved from defensive roles. Our results agree with this since the high chemical structural diversity of the pheromones suggest that the development of these signal chemicals were driven by environmental conditions instead of being family – driven. The structural diversity observed among the pheromone development of these beetles appears to be unaffected by family – specific evolution in that evidence of a parallel feature is not available.

The different beetle families evolved at different periods wherein Melolonthinae was more primitive than Rutelinae and Dynastinae. Despite of varying evolutionary times, a similar or unifying structural feature among the pheromones is not evident. In support, the results of the cluster analysis showed that the pheromone structural diversity was high. Hence it becomes more apparent that the geographical dispersion of the beetles may have led to the observed chemical diversity. This is possible since several beetle species from the same family are located in various geographical locations which can potentially mean that their predators are different. If the predators of these beetle species within the same family are different, then the chemicals they secrete for defensive purposes will be different too. It is also plausible that the pheromones evolved in response to predators that use these chemicals as signals to locate their prey. The high structural diversity points out that the there exists high diversity of predators that prey on these beetles are also very different. Aside from being a communication tool, pheromones also function as a predator's locator for a prey. It has been reported that some predators can utilize pheromones to track down their prey.

There are some predatory insect species that easily locate their targets through the use the prey's own pheromones, which are called kairomones (Wyatt 2003). An example of this the clerid beetle, Thanasimus dubious, that uses a method wherein its receptors are already set to the aggregation pheromone of its prey the pine beetle, Dedroctonus frontalis (Payne et al. 1984). Predators locating prey using their pheromones also results to a higher predation rate on mature adults during breeding season since pheromones are their main communication signal (Conover 2007).

In relation to chemotaxonomy, it is evident that using pheromones to establish phylogenetic relationships among the beetles by subfamily is very difficult. This is true even though the sex pheromones of Dynastinae and Melolonthinae both utilize derivatives with the former focusing on fatty acid and the latter on amino acid as well as terpenoid compounds (Leal 1971). An example of this is the Holotrichia parallela, a Melolonthinae beetle and is also known as large black chafer, which has a recognized L-isoleucine methyl ester as its major sex pheromone component (Leal

1971). In contrast, much is not known about the pheromones of beetles under the subfamily Dynastinae and only the Orycytes monoceros have identified compounds. Ethyl 4-methyloctanoate was found to be a compound of the aggregation pheromones of the mentioned Dynastinae beetle (Leal 1998). On the other hand, the Anomala rufocuprea, a Ruteline beetle, has japonilure and methyl 5-(Z)-tetradecenoate, which are oleic acid derivatives (Leal, 1998). Moreover, Leal (1971, 1998) stated that despite the utilization of similar pheromone blends of organisms of the same genus—for example, the Anomala cuprea, Anomala octiescostata, and Anomala albopilosa saksihama—a cross-attraction does not exist because of geographical or seasonal isolation. Overall, pheromones of Scarabaeoidea do not exhibit a distinct and unifying characteristic unlike for example, mealybugs. The pheromone structure of mealybugs and scale insects possess a common structural feature which is a long carbon chain with a terminal ester moiety (Zou and Millar 2015).

Conclusion

In conclusion, we have analyzed the structural diversity of scarab beetle pheromones using the Tanimoto coefficient. The results of the comparison revealed that the structural diversity was very high even among similar families. The implications of the observed high chemical diversity in chemotaxonomy suggest that pheromones cannot be used to classify these species. From an ecological perspective, the high structural diversity provides insights on the predators of the beetles. The high chemical diversity of pheromones even within the same family is indicative that the diversity of predators that feed on the beetles are also high since pheromones are also being used by predators to track down their prey. From an evolutionary perspective, the observed structural diversity suggests provides support from an earlier hypothesis suggesting that pheromone communication in scarab beetles evolved from defensive roles. Moreover, we also suggest an alternative hypothesis that pheromone communication evolved as a consequence of high predator diversity since pheromones are also used by predators to track their prey. The information and ideas presented in this study should be useful in further studying the behavior, ecology, and biochemistry of this class of beetles.

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